

1. (Currently Amended) A method of analyzing prokaryotic gene expression comprising the processes of:

an removing ribosomal RNA-mRNA isolation process for isolating an mRNA-from total RNA obtained from a prokaryotic cell to obtain a fraction of the total RNA enriched in mRNA;

adding a polyadenylation sequence addition process for adding a polyA at the 3' end of the isolated mRNA to obtain polyA-mRNA;

synthesizing a cDNA synthesis process for synthesizing a cDNA-from the polyA-added mRNA;

attaching a cDNA processing process for preparing an adaptor attached cDNA fragment having the sequence of a first adapter polynucleotide sequence at one end of the cDNA and the sequence of a second adapter polynucleotide sequence at the other end of, from the cDNA to obtain an adapter-attached cDNA;

amplifying a first PCR process for performing PCR with the adaptor-adapter-attached cDNA fragments, in a polymerization chain reaction (PCR) with using a first primer having a sequence complementary to the a sequence of the first adaptor adapter and a second primer having a sequence complementary to the a sequence of the second adaptor adapter;

isolating and recovering the amplified cDNA; and

analyzing prokaryotic gene expression with the recovered amplified cDNA an electrophoresis process for performing electrophoresis with the cDNA fragments amplified in the first PCR process; and

a cDNA fragment recovery process for recovering the desired cDNA fragment based on the result of the electrophoresis.

2. (Currently Amended) A method of analyzing prokaryotic gene expression The method according to Claim 1, wherein isolating the mRNA isolation process comprises:

a process of isolating the whole RNA from the prokaryotic cell;

<u>least a portion of 16S rRNA</u> with the 16S rRNA, and simultaneously hybridizing a second <u>poly</u>nucleotide having a sequence complementary to a portion of 23S rRNA with the 23S rRNA;

a process of hybridizing a third polynucleotide which is coupled to a first tag substance Tag Substance 1) to which is added a third nucleotide having wherein the third polynucleotide comprises a sequence complementary to the first polynucleotide at a site that is different from the site complementary in which the first polynucleotide is complementary to the 16S rRNA in the first nucleotide, with the hybrid of the 16S rRNA and the the first nucleotide thereby forming 16s rRNA hybrid molecules, and

simultaneously hybridizing a second tag substance to which is added a fourth polynucleotide which is coupled to a second tag substance wherein the fourth polynucleotide comprises having a sequence complementary to the second polynucleotide at a site that is different from the site complementary in which the second polynucleotide is complementary to the 23S rRNA in the second nucleotide, with the hybrid of the 23S rRNA and the second nucleotide thereby forming 23S rRNA hybrid molecules; and

and second tag substances hybrid of the 16S rRNA, the first nucleotide and the first tag substance added with the third nucleotide, and simultaneously removing the hybrid of the 23S rRNA, the second nucleotide and the second tag substance added with the fourth nucleotide, from the whole RNA.

3. (Currently Amended) A method of analyzing prokaryotic gene expression—The method according to Claim 2, wherein

the first polynucleotide and the second polynucleotide are identical ones having and comprise a sequence complementary to the a common sequence present in both 16S rRNA and 23S rRNA,

the third <u>poly</u>nucleotide and the fourth <u>poly</u>nucleotide are also identical, and the first tag substance and the second tag substance are also identical.

4. (Currently Amended) A method of analyzing prokaryotic gene expression The method according to Claim 1, wherein

synthesizing a cDNA further comprises the cDNA synthesis process comprises the synthesis of the cDNA as well as the addition of adding a tag substance at to the 5' end of the cDNA at the same time as the cDNA is synthesized; and

the cDNA processing process comprises:

a first cleavage process for cleaving wherein after the cDNA is synthesized, the cDNA by means of is cleaved with a type I restriction enzyme;

a first recovery process for recovering the <u>tagged cDNA</u> fragments having the tag substance by binding with a high-affinity substance having high affinity to the tag substance;

a binding process of wherein the first adaptor adapter for binding to the cDNA fragments having the tag substance, the sequence of the first adaptor having comprises a sequence complementary to the sequence at the cleavage site of the type I restriction enzyme;

a second cleavage process for cleaving the cDNA fragment bonded with the sequence of attached to the first adapter by means of with a type II restriction enzyme;

a second recovery process for removing the cDNA fragments with the tag substance and recovering the cDNA which does not have fragment not having the tag substance, by binding them with the high-affinity substance; and

a binding process of wherein the second adapter adapter for binding to the cDNA fragment not having the tag substance, the sequence of a second adaptor having comprises a sequence complementary to the sequence at the cleavage site of the type II restriction enzyme.

5. (Currently Amended) A method of analyzing prokaryotic gene expression The method according to Claim 1, wherein

the electrophoresis process is carried out by means of isolating and recovering the amplified cDNA comprises subjecting the amplified cDNA to gel electrophoresis; and

the cDNA fragment recovery process is carried out by recovering the amplified cDNA by cutting out the a portion of the gel containing the desired cDNA fragment from the gel and recovering the corresponding cDNA from the gel fragment.

- 6. (Currently Amended) A method of analyzing prokaryotic gene expression—The method according to Claim 1 Claim 5, wherein at least one part of the first primer and the second primer is(are) given with are labeled with a marker substance, and wherein the marker substance is detected detectable in the gel electrophoresis.
- 7. (Currently Amended) A method of analyzing prokaryotic gene expression—The method according to Claim 4, wherein the combination of the tag substance and the high-affinity substance is any one of the combinations a combination of biotin and streptavidin, of biatin biotin and avidin, of FIGT and FITI antibody, and of DIG and anti-DIG.

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8. (Currently Amended) A method of analyzing prokaryotic gene expression The method according to Claim 1, which <u>further</u> comprises, after <u>recovering</u> the cDNA <u>fragment</u> recovery process,

a ligation process for ligating the recovered cDNA fragment to a plasmid vector to form a recombinant plasmid; and

an incorporation process for incorporating transforming an *Escherichia coli* cell with the recombinant plasmid into Escherichia coli.

9. (Currently Amended) A method of analyzing prokaryotic gene expression The method according to Claim 8, which further comprises, after recovering the cDNA fragment recovery process and before the ligation process ligating the recovered cDNA into a plasmid vector, a second PCR process for performing PCR with the amplifying the recovered cDNA fragment, using with a third primer having a sequence complementary to the sequence of the first adapter and a fourth primer having a sequence complementary to the sequence of the second adapter adapter.